Team iGEM UFSCar-Brasil

# POLYMERASE CHAIN REACTION (PCR)

1. Add the components in a sterile PCR tube in this following order and keep it on ice.

| COMPONENT | 50 µL REACTION | FINAL CONCENTRATION |
|---|---|---|
| Nuclease-free water | 42.35 µL | - |
| Buffer 10X | 5 µL | 1X |
| dNTP 10mM | 0.5 µL | 0.1 mM |
| Forward Primer 10 µM | 0.5 µL | 0.1 µM |
| Reverse Primer 10 µM | 0.5 µL | 0.1 µM |
| DNA 2 ng/µL | 1 µL | 40 pg/µL |
| *Taq* DNA Polymerase 5 U/µL | 0.25 µL | 25 mU/µL |
| Total | 50 µL | - |

2. Transfer PCR tubes from ice to a thermocycler, start the PCR reaction.

3. Thermal cycling protocol:

| STEP | TEMPERATURE | TIME | NUMBER OF CYCLES |
|---|---|---|---|
| Initial denaturation | 95 ºC | 3 minutes | 1 |
| Denaturation | 94 ºC | 30 seconds | 35 |
| Annealing | 50 ºC | 30 seconds | |
| Extension | 72 ºC | 1 minute | |
| Final extension | 72 ºC | 5 minutes | 1 |
| Keep | 4 ºC | ∞ | - |

4.Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining.